Rerouting trafficking circuits through posttranslational SNARE modifications

Warner, Harry; Mahajan, Shweta; van den Bogaart, Geert

Published in:
Journal of Cell Science

DOI:
10.1242/jcs.260112

© 2022. Published by The Company of Biologists Ltd | Journal of Cell Science (2022) 135, jcs260112. doi:10.1242/jcs.260112

REVIEW

SUBJECT COLLECTION: MEMBRANE TRAFFICKING

Rerouting trafficking circuits through posttranslational SNARE modifications

Harry Warner[1], Shweta Mahajan[2] and Geert van den Bogaart[1,*]

ABSTRACT

Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are membrane-associated trafficking proteins that confer identity to lipid membranes and facilitate membrane fusion. These functions are achieved through the complexing of Q-SNAREs with a specific cognate target R-SNARE, leading to the fusion of their associated membranes. These SNARE complexes then dissociate so that the Q-SNAREs and R-SNAREs can repeat this cycle. Whilst the basic function of SNAREs has been long appreciated, it is becoming increasingly clear that the cell can control the localisation and function of SNARE proteins through posttranslational modifications (PTMs), such as phosphorylation and ubiquitylation. Whilst numerous proteomic methods have shown that SNARE proteins are subject to these modifications, little is known about how these modifications regulate SNARE function. However, it is clear that these PTMs provide cells with an incredible functional plasticity; SNARE PTMs enable cells to respond to an ever-changing extracellular environment through the rerouting of membrane traffic. In this Review, we summarise key findings regarding SNARE regulation by PTMs and discuss how these modifications reprogramme membrane trafficking pathways.

KEY WORDS: Membrane traffic, SNARE proteins, Posttranslational modifications

Introduction

Membrane traffic between organelles is a carefully choreographed process requiring the sculpting and transport of both vesicles and tubules. Following transport, vesicles and tubules must fuse with organelle membranes. Critical to these processes are the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs). Vesicles cannot fuse in the absence of SNAREs (Weber et al., 1998), and there is increasing evidence that, in addition to regulating membrane–membrane fusion, SNARE interactions with non-SNARE proteins can regulate vesicle biogenesis and mobilisation (Sneeggen et al., 2019). SNAREs drive membrane fusion through the binding of R-SNAREs (which are mostly localised to vesicles) to Q-SNAREs (on organelle membranes). Typically, one R-SNARE on a vesicle will complex with three Q-SNAREs (termed Qa, Qb and Qc; Dingjan et al., 2018; Jahn and Scheller, 2006). The exception are soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) family SNARE proteins, which carry both Qb and Qc SNARE motifs (Jahn and Scheller, 2006). Each set of Q-SNAREs is thought to complex with a limited number of R-SNAREs, providing membranes with a SNARE-associated identity (Dingjan et al., 2018; Söllner et al., 1993). SNAREs are known to be loaded onto their associated membranes by vesicle coat components (Matsuoka et al., 1998). Following membrane fusion, SNARE complexes are disassembled by the N-ethylmaleimide-sensitive factor (NSF) protein (Block et al., 1988; Malhotra et al., 1988), along with the cofactor α-SNAP (also known as NAPA; see Fig. 1 for a basic overview of SNARE function). However, it is becoming clear that SNARE proteins are extensively regulated via posttranslational modifications (PTMs). This provides SNARE proteins with functional fluidity; PTMs may alter the ability of SNARE proteins to fuse membranes and can generate subfamilies of SNARE-based vesicle identities. This may be critical for enabling cells to adjust to an ever-changing environment. For instance, it is already known that stimulation of epithelial cells with growth factors or of immune cells with pathogenic stimuli requires rerouting of membrane trafficking circuits (Francavilla et al., 2013; 2016; Verboogen et al., 2019). Such events may also be (partially) achieved by posttranslationally modifying SNARE proteins; in fact, in dendritic cells this has been demonstrated (Nair-Gupta et al., 2014) (see below). Thus, SNARE proteins, along with phosphoinositides and Rab proteins, can be altered to generate vast numbers of vesicular subclasses, both in basal conditions and in response to environmental cues. Here, we will provide an overview of the different PTMs known to reprogramme SNARE function. We will focus on phosphorylation, ubiquitylation, SUMOylation, palmitoylation, acetylation and O-GlcNAcylation, as these are well understood. However, it is likely that a myriad of other PTMs (for example, methylation) also regulate SNARE function. We will give a comprehensive description of how these SNARE PTMs can alter membrane traffic and will highlight outstanding questions or challenges in the field (for a comprehensive list of SNARE PTMs observed in high-throughput human and mouse datasets, mapped to the human modification sites, see Table S1; Hornbeck et al., 2015).

SNARE regulation through phosphorylation

The addition of a relatively small phosphate group to serine, threonine or tyrosine residues has long been known to reprogramme the function of proteins (Ardito et al., 2017). Furthermore, the small size (95 Da) and negative charge (−2 at physiological pH) of a phosphate group have allowed the development of effective phosphopeptide enrichment strategies, which, when combined with mass spectrometry, enable the monitoring of global changes in phosphorylation states under varying cellular conditions (Fílla and Honys, 2012). SNARE proteins are also subject to regulation via phosphorylation, although the underlying details of this process are only just emerging. Indeed, several phosphoproteomic datasets (Olsen et al., 2010; Matsuoka et al., 2007; Sharma et al., 2014) have identified different SNARE phosphorylation sites under specific

[1]Department of Molecular Immunology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747AG Groningen, The Netherlands. [2]Division of Immunobiology, Center for Inflammation and Tolerance, Cincinnati Children's Hospital, Cincinnati, OH 45229, USA.

*Author for correspondence (g.van.den.bogaart@rug.nl)

G.v.d.B., 0000-0003-2180-6735

Journal of Cell Science (2022) 135, jcs260112. doi:10.1242/jcs.260112

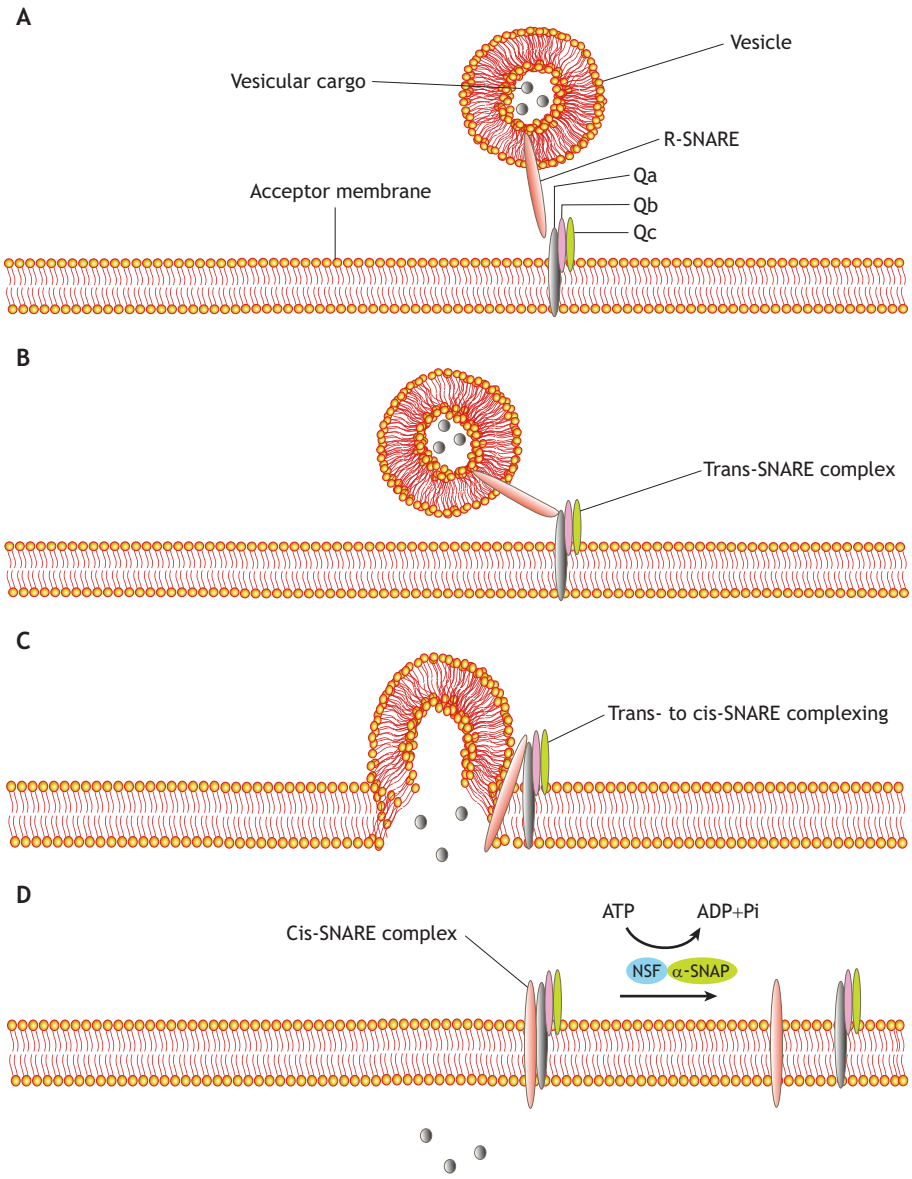

Fig. 1. General model of SNARE-mediated membrane fusion. (A) Vesicles with associated R-SNAREs are trafficked to acceptor membranes (such as the Golgi or plasma membrane), which contain a Q SNARE cluster composed of a Qa-SNARE, Qb-SNARE and Qc-SNARE. (B) N- to C-terminal zippering is initiated between the vesicular R-SNARE and the Q-SNARE complex. (C) Zippering between the R-SNARE and Q-SNAREs drives the fusion of the vesicular membrane with the acceptor membrane, enabling the content of the vesicle to pass across the acceptor membrane. (D) The resulting cis-SNARE complex is disassembled by NSF and α-SNAP in an ATP-dependent manner. This provides the free energy for future membrane fusion events. A single SNARE complex is sufficient to drive membrane–membrane fusion. Pi, phosphate.

conditions, but many of their functions are obscure. However, it is currently understood that SNARE phosphorylation can alter both the fusogenic capacity of SNAREs, as well as the identity they confer. Therefore, we will summarise key recent studies on how phosphorylation rewires membrane traffic, providing (where possible) the precise molecular explanation of how phosphorylation achieves these ends.

In the context of immune cell biology, a critical role for phosphorylation of the SNARE protein SNAP23 (a Q-SNARE) in antigen cross-presentation in mouse bone marrow-derived dendritic cells has been found (Nair-Gupta et al., 2014). Dendritic cells are the interface between the innate and adaptive immune systems; they phagocytose and digest pathogens before trafficking pathogen-derived peptides (referred to as antigens) bound to major histocompatibility complexes (MHCs) to the plasma membrane (Patente et al., 2019). MHC-I (which mediates antigen presentation to CD8-positive T cells in a process referred to as cross-presentation) is stored in the endocytic recycling compartment (ERC) in a Rab11a-dependent manner to be delivered to the phagosome following the ingestion of microbial pathogens. Thus, prior to encountering a pathogen, trafficking networks prime the cell for a rapid response to ensure rapid antigen cross-presentation. Indeed, in mice, myeloid differentiation primary response 88 (MyD88)-dependent toll-like receptor (TLR) signalling in response to inflammatory cargo from bacterial pathogens induces SNAP23 phosphorylation on serine 95 (which is conserved in human SNAP23) at the phagosome, triggering the trafficking of MHC-I to the phagosome (Nair-Gupta et al., 2014). It is still unclear how this phosphorylation enables cross-presentation, but it is possible that it allows SNARE complex formation (likely between SNAP23 and VAMP8, but this remains unconfirmed). This seems to be a likely explanation, as SNAP23 phosphorylation has been shown to stabilise SNARE complexes in mast cells (Karim et al., 2013; Puri and Roche, 2006; Suzuki and Verma, 2008). Thus, mouse dendritic cells prime their trafficking machinery prior to encountering a pathogen and utilise the phosphorylation of SNAP23 as a hair trigger to rapidly initiate MHC-I shuttling to the phagosome (Nair-Gupta et al., 2014).

Furthermore, all non-synaptic R-SNAREs (a subfamily of R-SNAREs that are absent in neuronal synapses) have conserved

Journal of Cell Science (2022) 135, jcs260112. doi:10.1242/jcs.260112

serines and threonines that potentially can be phosphorylated to alter their fusogenic capacity (Malmersjö et al., 2016). These sites are found inside the helical bundle that is formed when R-SNAREs bind to Q-SNAREs. It has been further demonstrated that introducing phosphomimetic mutations to the R-SNARE VAMP8 at one of three conserved residues (T47E, T53E or S54E – all of which are conserved between mouse and human) can block *in vitro* SNARE complex formation. Furthermore, in rat mast cells expressing these VAMP8 mutants, vesicles can dock at the plasma membrane, but they fail to fuse. Thus, although phosphorylated VAMP8 might still be able to form a complex with its cognate SNAREs (i.e. incomplete binding that does not promote membrane–membrane fusion), the SNARE complex cannot fully form and vesicle–membrane fusion is blocked by R-SNARE phosphorylation (Fig. 2) (Malmersjö et al., 2016). This therefore points towards a model whereby, in cells such as mast cells, VAMPs may be phosphorylated to enable the accumulation of vesicles at the plasma membrane that are primed for exocytosis, possibly by dephosphorylation (Fig. 2A). This might allow cells to rapidly release the content of these vesicles (such as histamine or heparin) into the extracellular space when required (such as when encountering a pathogen-bound IgE antibody; Galli and Tsai, 2012) (Fig. 2B). In line with this model, mutations located at the C-terminal end of the SNARE region of VAMP2 still allow formation of a trans-SNARE complex but impair membrane fusion, because the complete cis-SNARE complex cannot form (Hernandez et al., 2012). Hence, it might be expected that PTMs located at the C-terminal end of the SNARE regions might enable membrane tethering but stall fusion. In this case, removal of the PTM might result in cis-SNARE complex formation and so trigger membrane fusion. However, this mechanism still needs to be confirmed *in vivo*.

A role for STX17 phosphorylation in the regulation of autophagy has been described in human cell lines and mouse bone marrow-derived macrophages (Kumar et al., 2019). Typically, STX17 is thought to be involved in membrane trafficking between the endoplasmic reticulum (ER) and the Golgi (Muppirala et al., 2011). However, the function of STX17 can be reprogrammed in order to facilitate autophagy (Kumar et al., 2019), which is achieved through TANK-binding kinase 1 (TBK1)-mediated phosphorylation at serine 202 (Fig. 3A) (within the target SNARE coiled-coil homology motif, which is conserved between mouse and human). This leads to STX17 recruitment to the Golgi (Fig. 3B). Upon induction of autophagy by starvation, phosphorylation of STX17 results in its departure from the Golgi and drives the formation of the pre-autophagosomal structure (PAS), permitting the assembly of autophagosomes (Fig. 3C). How the precise location of autophagosome assembly is dictated is unknown; however, there is strong evidence that this occurs at an ER–mitochondrial contact site (Hamasaki et al., 2013). The assembly of the mammalian PAS is achieved through the interaction of phosphorylated STX17 with ATG13 and FIP200 (also known as RB1CC1), which in turn recruit ULK1. Autophagy is blocked in STX17-knockout cells,

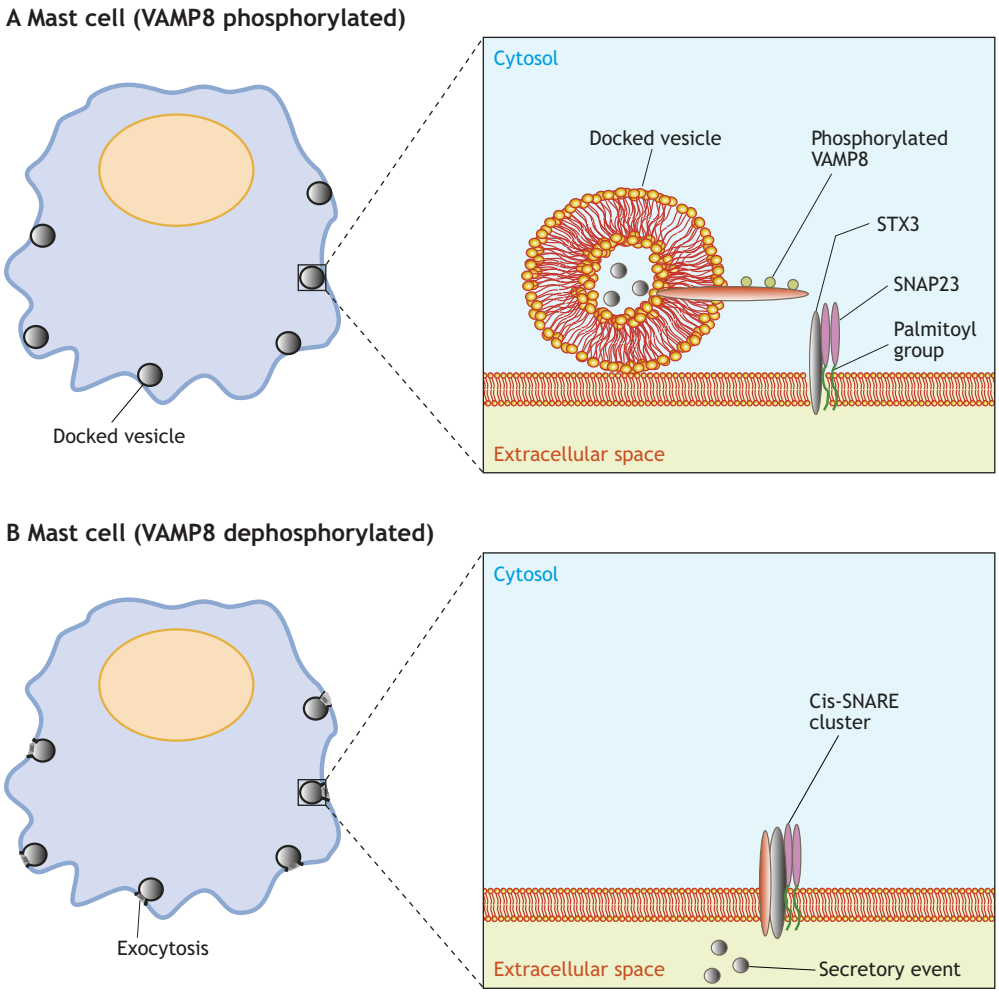

Fig. 2. Model for mast cell degranulation through VAMP8 dephosphorylation. (A) Under non-inflammatory conditions, mast cells maintain vesicles at the plasma membrane that are filled with exocytic cargoes. Although docked, these vesicles are unable to fuse because phosphorylation of VAMP8 blocks its complex formation with STX3. (B) Following inflammatory stimulation, VAMP8 is dephosphorylated by a phosphatase, enabling its interaction with STX3 at the plasma membrane; this drives vesicle fusion and release of vesicular content to further trigger the immunological response.

Journal of Cell Science (2022) 135, jcs260112. doi:10.1242/jcs.260112

and this can be rescued by the overexpression of wild-type STX17 or the phosphomimetic mutant STX17 S202E, but not by the phosphodead mutant STX17 S202A, demonstrating that phosphorylation of STX17 at serine 202 is essential for autophagy. In summary, STX17 can be shuttled between biosynthetic and autophagic trafficking pathways through the dynamic phosphorylation and dephosphorylation of serine 202 (Hamasaki et al., 2013).

Two recent studies have identified conserved phosphorylation events on the R-SNARE YKT6 (Pradhipa Karuna et al., 2020; McGrath et al., 2021). YKT6 is an unusual SNARE, because it is extensively regulated through palmitoylation, geranylgeranylation and farnesylation. YKT6 has been described as a regulator of ER-to-Golgi traffic. The first of these recent studies now demonstrates that phosphorylation is a central regulator of YKT6-dependent ER-to-Golgi traffic and that, conversely, dephosphorylation can reprogramme the YKT6 interactome in order to drive autophagy. Specifically, a role for YKT6 phosphorylation in driving YKT6 opening and recruitment to membranes in general has been shown (YKT6 is normally found in a closed cytosolic form) (Pradhipa Karuna et al., 2020). Here, four phosphorylation sites (including serine 174) are shown to be conserved between *Drosophila* and humans. Expression of phosphomimetic or phosphodead (all four serines converted) YKT6 mutants in YKT6$^{-/-}$ *Drosophila* points to a physiological role for these sites, as wild-type YKT6 and the phosphodead mutant reverse the lethal phenotype caused by the knockout, whereas the phosphomimetic mutant fails to reverse it. The authors further show that phosphorylation of these serines drives an open conformation and reprogrammes the interactome of YKT6 (as described using mass spectrometry); however, the overall consequences of this are yet to be defined (Pradhipa Karuna et al., 2020).

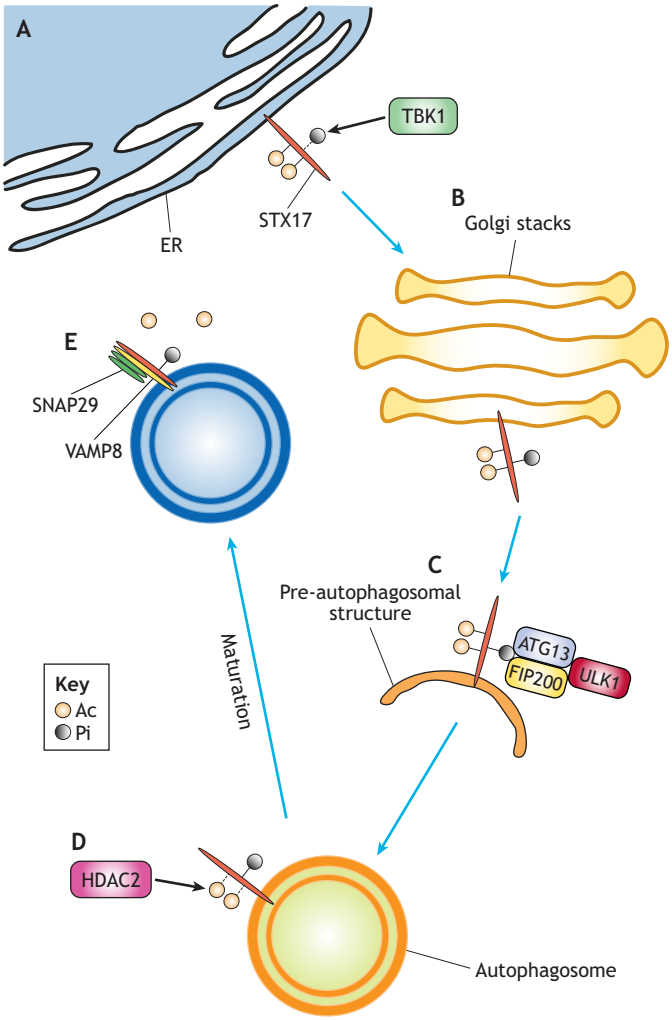

Fig. 3. Model of STX17 regulation during autophagy. (A) Constitutively acetylated STX17 localised at the ER must be phosphorylated by TBK1 at serine 202. (B) Phosphorylation of STX17 relocates it to the Golgi, from where it can be recruited to the pre-autophagosomal structure (likely at an ER–mitochondrial contact site). (C) At the pre-autophagosomal structure, phosphorylated STX17 can recruit ATG13, FIP200 and ULK1, driving the formation of an autophagosome. (D) HDAC2 deacetylates STX17 at the autophagosomal membrane. (E) Deacetylated STX17 is able to form a complex with VAMP8 and SNAP29, which drives autophagosomal maturation through fusion of the autophagosome with lysosomes to ensure the degradation of the autophagic cargo. Ac, acetyl group; Pi, phosphate.

The second study examines serine 174 of YKT6 (serine 176 in the yeast orthologue Ykt6). Specifically, the authors also demonstrate that this residue must be phosphorylated in order for YKT6 to switch from a closed, cytosolic form to an open, membrane-bound form (McGrath et al., 2021). They show that this phosphorylation occurs in response to Ca^{2+} signalling and is required for the role of YKT6 in trafficking along the secretory pathway, from the ER and Golgi, in human cells. They also show using mass spectrometry that the phosphodead (closed cytosolic) variant of YKT6 interacts more strongly with the Q-SNAREs SNAP29 and STX17 (SNAREs required for autophagy), compared to the phosphomimetic mutant. Consistent with this, overexpression of wild-type Ykt6 and an S176A Ykt6 mutant, but not a S176D mutant, in yeast was protective against the toxic effects of misfolded α-synuclein (a driver of Parkinson's disease). This effect presumably occurs because wild-type Ykt6 and the phosphodead Ykt6 mutant can still interact with autophagy-associated SNAREs to degrade misfolded α-synuclein, but the phosphomimetic mutant cannot. However, this remains to be confirmed through colocalisation experiments (McGrath et al., 2021). Based on these data, it seems possible that phosphorylation of YKT6 at serine 174 is central to shuttling YKT6 between the secretory pathway and the autophagy pathway, although more work will be required to verify this. There are also many open questions regarding YKT6 regulation that still need to be addressed.

Interestingly, a role for dephosphorylation of the Q-SNARE VTI1b in enabling autophagy has also been recently described (Chou et al., 2021). Using a genetic screen in *Drosophila* to discover phosphatases regulating autophagy, the authors identified the phosphatase Ptpmeg2, which is a homologue of mammalian PTPN9. When PTPN9 was knocked down in mouse cells, the number and size of autophagy related 16 like 1 (ATG16L1)-carrying vesicles (required for autophagosome formation) were found to be reduced. Q-SNARE VTI1b was shown to be a substrate of PTPN9, which dephosphorylates tyrosines 112 and 115 of VTI1b. Expression of the phosphomimetic Y112E Y115E VTI1b mutant (which has both tyrosines converted to glutamic acids) was found to block autophagy. Conversely, overexpression of the phosphodead Y112F Y115F VTI1b mutant rescued the reduction in number and size of ATG16L1-positive vesicles observed in PTPN9-knockdown cells. Whilst the exact mechanism underpinning autophagy initiation by VTI1b dephosphorylation is unclear, it seems likely that the phosphate groups block interaction with relevant R-SNAREs. Thus, VTI1b is constitutively phosphorylated to block autophagy but can be reprogrammed by Ptpmeg2 in

Journal of Cell Science (2022) 135, jcs260112. doi:10.1242/jcs.260112

Drosophila, or by PTPN9 in mammals, to promote autophagy when required (Chou et al., 2021).

In summary, SNARE phosphorylation events can promote and block SNARE-driven membrane fusion, as well as reprogramme SNARE interactomes. Thus, kinases and phosphatases can act as interfaces between extracellular stimuli and SNARE proteins to redirect membrane traffic, achieving a diverse array of cellular outcomes, including antigen cross-presentation, autophagy and exocytosis.

SNARE ubiquitylation and SUMOylation

Ubiquitylation

Ubiquitylation, originally identified as a label for proteasomal degradation, is now appreciated as a complex PTM that is capable of regulating a wide variety of cellular events, including membrane traffic (Kliza and Husnjak, 2020). During ubiquitylation, a ubiquitin molecule is transferred from an E2 ubiquitin-conjugating enzyme to a target protein via an E3 ubiquitin ligase (Kliza and Husnjak, 2020). Ubiquitin monomers are very small (76 residues) and can readily be identified through a number of proteomic techniques, and several SNARE ubiquitylation events have been identified. Although the function of many of these ubiquitylations remains elusive, a few key studies have shed light on how they can reprogramme SNARE function.

A role for ubiquitylation of the Q-SNARE STX5 in the p97 (VCP)-dependent pathway of mitotic Golgi fragmentation has been identified (Huang et al., 2016). During interphase, the Golgi structure is maintained by fusion events driven by a complex between STX5 and the R-SNARE BET1 (Fig. 4A). A search for substrates of the E3 ubiquitin ligase HACE1 with known roles in Golgi fragmentation during mitosis identified STX5 as a mitotic ubiquitylation target (at K270 in the short isoform, K325 in the long isoform – present in both mouse and humans). Monoubiquitylation of STX5 at K270 blocks its interaction with BET1, driving Golgi fragmentation (because fusion reactions can no longer occur) (Fig. 4B). This ubiquitylation also enables STX5 to recruit p47 (also known as NSFL1C), the adaptor for both p97 (a protein that further drives Golgi fragmentation) and the deubiquitylase VCIP135 (also known as VCPIP1) (Fig. 4C). However, during mitosis, VCIP135 is kept inactive by a mitosis-dependent phosphorylation event, and as cells progress to late mitosis, VCIP135 is dephosphorylated and able to deubiquitylate STX5, enabling re-formation of the Golgi (Fig. 4D,E) (Huang, et al., 2016). This study not only shows how STX5 function is rewired in mitosis, as compared to interphase, by altering its fusogenic ability, but also points to an interplay between deubiquitylases and phosphatases that ultimately determines STX5 function.

Ubiquitylation also plays a role in the endocytic recycling of SNAREs. In the yeast *Saccharomyces cerevisiae*, the SNARE Snc1 (an R-SNARE required for fusion of exocytic vesicles formed at the Golgi) is polyubiquitylated on K63, and this polyubiquitylation is required for endocytic recycling of Snc1 (Xu et al., 2017). The ubiquitylation of Snc1 had already been known (Chen et al., 2011), but to determine its function, the deubiquitylase UL36 from herpes simplex virus was fused to GFP-tagged Snc1, which blocked Snc1 recycling and drove its accumulation on Tlg1-positive endosomes (Xu et al., 2017). Furthermore, the authors identified Tul1 and Pib1 as the E3 ubiquitin ligases responsible. In addition, polyubiquitylation was shown to drive an interaction between Snc1 and COPI coat proteins (normally found on the Golgi, but also observed on Tlg1-positive endosomes), specifically between the polyubiquitin group and the WD40 repeat propeller domains of the

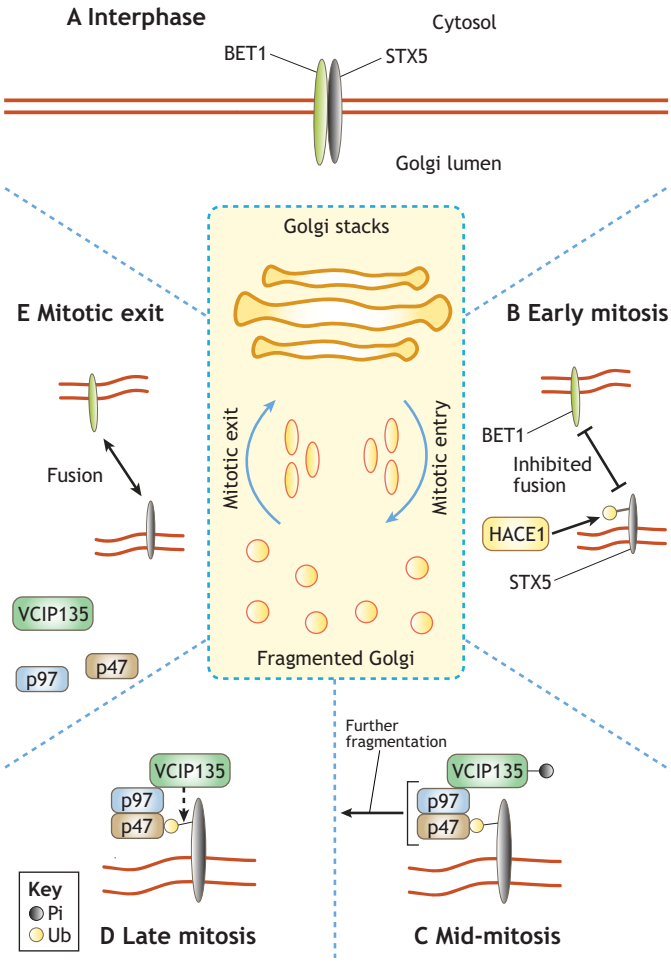

Fig. 4. Monoubiquitylation of STX5 is important for Golgi assembly and disassembly. (A) During interphase, STX5 and BET1 maintain Golgi stack structure through undergoing continuous fusion reactions. (B) During mitosis, STX5 is monoubiquitylated at lysine 270 by HACE1, which blocks the interaction with BET1, resulting in fragmentation of the Golgi membrane as fusion cannot take place. (C) Recruitment of p97 and p47 to ubiquitylated STX5 further drives Golgi fragmentation. p97 and p47 also recruit the deubiquitylase VCIP135 in a phosphorylated (inactive) state, which primes the p97–p47–STX5 complex for disassembly. (D) Dephosphorylation of VCIP135 upon mitotic exit activates its deubiquitylase activity, enabling deubiquitylation of STX5. (E) Removal of ubiquitin from lysine 270 in STX5 leads to the dissociation of p97, p47 and VCIP135 from STX5, which allows STX5 to interact with BET1, resulting in re-formation of the interphase Golgi structure. Pi, phosphate; Ub, ubiquitin.

β′-COP subunit (also known as Sec27; Xu et al., 2017). This interaction is essential for the endocytic recycling of Snc1 to the plasma membrane (via the Golgi). Thus, the polyubiquitylation of Snc1 is required for its recycling to the Golgi to enable further rounds of exocytosis. This ubiquitylation–deubiquitylation cycle therefore allows the cell to control the subcellular location of Snc1, perhaps in response to extracellular stimuli. However, this mechanism does not appear to be conserved in humans, as the ubiquitylation site is not conserved in VAMP2, the closest human orthologue of yeast Snc1 (38% sequence identity).

A further example of the regulation of SNARE localisation is ubiquitylation of the Q-SNARE STX3 in a conserved polybasic domain, which is required for the retrieval of STX3 from the basolateral domain of polarised epithelial cells (Giovannone et al., 2017). Using human cell lines, it has been demonstrated that this

ubiquitination can likely occur on any of the five C-terminal lysines found in STX3. In Madin–Darby canine kidney (MDCK) cells, this ubiquitylation directs STX3 and associated cargoes (such as the known exosomal cargo GPRC5B) into the endolysosomal system, which ultimately leads to their sequestration into intraluminal vesicles that are subsequently secreted as exosomes. Finally, experiments in the human foreskin fibroblast cell line BJ1 show that human cytomegalovirus (replication of which is dependent on STX3) hijacks this pathway, as cells expressing a nonubiquitylatable mutant (in which the five lysines are converted into arginines) produce fewer virus particles when infected (Giovannone et al., 2017). Although the exact underlying mechanism is unclear, this study nevertheless suggests that the ubiquitylation of STX3 is integrated into the epithelial polarity programme (as in MDCKs) and is used in cells with a mesenchymal polarity (such as BJ1 cells). Therefore, it will be interesting for future studies to examine whether ubiquitylation of STX3 is front–rear polarised in mesenchymal cells.

SUMOylation

SUMOylation is a PTM analogous to ubiquitylation and plays an important role in a wide range of cellular processes. SUMOylation involves the conjugation of a small ubiquitin-like modifier (SUMO) to lysine residues in target proteins (Henley et al., 2014; Wilkinson and Henley, 2010). SUMOylation has been characterised as a major regulator of numerous functions, including trafficking, synapse formation (Shalizi et al., 2006) synaptic plasticity (Chamberlain et al., 2012; Craig et al., 2012) as well as transcription and even chromosome segregation (Wilkinson and Henley, 2010). As with ubiquitylation, SUMOylation can regulate membrane traffic through modifying SNARE proteins, although the details of this are just emerging.

SUMOylation of the neuronal Q-SNARE STX1A regulates neurotransmitter release from the presynaptic terminal of rat neurons (Craig et al., 2016). During exocytosis at the presynaptic terminal, SNARE proteins including STX1A provide the mechanical force required for membrane fusion. Here, the authors demonstrate that STX1A is SUMOylated on lysines 252, 253 and 256 (which are conserved between rat and human), an event that is dependent on activation of the neuron. Preventing STX1A SUMOylation through mutagenesis of these residues leads to an increase in the rate of vesicle endocytosis, but the rate of exocytosis is unaffected. Thus, SUMOylation of STX1A acts as a molecular switch that coordinates the balance between endocytosis and exocytosis. Based on this, the authors propose that non-SUMOylated and SUMOylated STX1A form two distinct functional pools, with non-SUMOylated STX1A suggested to be involved in SNARE complex formation and membrane fusion, whereas SUMOylated STX1A dissociates from its cognate SNAREs and regulates vesicle endocytosis (Craig et al., 2016). These results demonstrate that the rapid membrane trafficking events that accompany neuronal activation are in part dependent on SNARE PTMs.

SUMOylation of STX1A has also been shown to regulate multiple steps of insulin secretion in a rat pancreatic β cell line (INS-1E; Davey et al., 2019). Specific inhibition of STX1A SUMOylation (using the same STX1A mutant as in Craig et al., 2016: STX1A K252R K253R K256R) leads to an increase in glucose-stimulated insulin secretion (GSIS). Thus, SUMOylation of STX1A acts as a brake on GSIS (Davey, et al., 2019). The underlying mechanism is unclear, but it is likely that SUMOylation blocks the formation of fusogenic SNARE complexes at the plasma membrane, preventing the exocytosis of insulin. This is in contrast to neurons, in which exocytosis is not blocked by STX1A SUMOylation per se. Thus, SUMOylation of STX1A is a critical regulatory node that might enable pancreatic β cells to dynamically modulate insulin secretion in accordance with the blood glucose concentration. It is unclear, however, why this SUMOylation event has different effects in neurons compared to pancreatic β cells; it is likely that SUMOylation alters trafficking in accordance with tissue-specific expression patterns of trafficking machinery.

In summary, both ubiquitylation and SUMOylation can redirect membrane traffic through modifying SNAREs. Furthermore, in contrast to phosphorylation (an ‘on–off’ modification), it is tempting to speculate that conjugated ubiquitin or SUMO groups might be further modified in additional ubiquitylation or SUMOylation steps. This suggests that ubiquitin or SUMO groups could potentially reprogramme SNARE function in a rheostat-like manner. This would allow for the dynamic modification of SNARE proteins, giving rise to specific new ubiquitylation and SUMOylation patterns (a ubiquitin and SUMO pattern code akin to the histone acetylation code; Prakash and Fournier, 2017) in response to an ever-changing extracellular environment.

SNARE palmitoylation, acetylation and O-GlcNAcylation

Palmitoylation

S-palmitoylation is the attachment of palmitate, a 16-carbon saturated fatty acid chain, to cysteine residues of target proteins by thioester linkages (typically by palmitoyltransferases) and is frequently involved in membrane association and protein sorting (Linder and Deschenes, 2007). In fact, numerous SNAREs – including SNAP23, SNAP25, STX7 and STX11 – have to be palmitoylated to exert their function (Dingjan et al., 2018). However, it is becoming clear that other SNAREs can be transiently palmitoylated in order to switch functions.

In yeast, Swf1 has been identified as the palmitoyltransferase responsible for palmitoylating the SNAREs Snc1, Syn8 and Tlg1 at cysteine residues close to or within their transmembrane domains (Valdez-Taubas and Pelham, 2005). The authors initially identified Swf1 through immunoblotting for Snc1 expressed in yeast strains deficient for different members of the DHCC-CDR family of putative palmitoyltransferases. Furthermore, palmitoyl-deficient Tlg1 was found to be missorted and degraded owing to targeting of unpalmitoylated Tlg1 by the ubiquitin ligase Tul1, which leads to the sorting of Tlg1 into multivesicular bodies that are ultimately degraded in the vacuolar compartment. These findings indicate that palmitoylation is necessary for proper SNARE targeting and maintenance. It is unknown whether this mechanism is conserved in humans, although this might be the case as the human orthologues of Snc1, Syn8 and Tlg1 – VAMP2, STX8 and STX10, respectively – all contain cysteine residues within their transmembrane helices or in their membrane-juxtaposed linker regions (Bateman et al., 2021). Palmitoylation may therefore act as a form of quality control or as a way to supress a trafficking circuit that is dependent on these SNAREs.

Another example of how S-palmitoylation affects SNARE functions concerns STX7 and STX8 (He and Linder, 2009). Both STX7 and STX8 function within the endosomal trafficking network but have been suggested to have distinct trafficking functions (Prekeris et al., 1999). However, there are many questions with regards to their precise function. In HeLa cells, palmitoylation occurs at cysteine 239 and cysteine 214 of STX7 and STX8, respectively (both of which are conserved between mouse and human) (He and Linder, 2009). Furthermore, STX7 and STX8 palmitoylation display differential sensitivity to brefeldin A (BFA; a

fungal metabolite that blocks transport through the secretory pathway); BFA substantially inhibits palmitoylation of STX7, whereas that of palmitoylation STX8 is only slightly decreased. Based on these observations, the authors suggest that palmitoylation of STX7 and STX8 occurs on different subcellular compartments – presumably post- and pre-Golgi. Interestingly, palmitoylation does not affect STX7 or STX8 turnover rates. Furthermore, abolishing palmitoylation does not influence the steady-state localisation of STX8 at late endosomes, whereas palmitoylation is important shuttling STX7 between the plasma membrane and endosomes (He and Linder, 2009). Although the implications of these results are unclear for STX8, these results suggest that the subcellular location of STX7can be determined through dynamic palmitoylation and depalmitoylation events, providing another potential interface between cell signalling events and the trafficking machinery by modifying trafficking circuits accordingly.

A role for palmitoylation in the control of STX19 at multiple levels has been described (Ampah et al., 2018). In mammalian cells, STX19 is palmitoylated on multiple cysteines within a conserved C-terminal cysteine-rich domain by members of the zDHHC acyltransferase family at the Golgi. These palmitoylations recruit STX19 to tubular recycling endosomes and block its degradation by the proteasome. Furthermore, palmitoylation of STX19 can drive the repositioning of Rab8-positive vesicles from intracellular organelles (the identities of which are not established in the study) to the plasma membrane (Ampah et al., 2018). These results suggest that rerouting of SNAREs can have far-reaching effects on other aspects of membrane traffic by fundamentally reprogramming cellular behaviour.

Finally, it has recently been shown that S-palmitoylation of neuronal SNARE STX1A regulates the spontaneous exocytosis of synaptic vesicles in murine cultured hippocampal neurons (Vardar et al., 2022). STX1A is palmitoylated at cysteine residues 271 and 272 located in its transmembrane domain (both of which are conserved in human) (Kang et al., 2008). The conversion of these residues to valines (a palmitoylation-dead mutant) results in a decreased spontaneous vesicle fusion (Vardar et al., 2022). Moreover, the authors report that palmitoylation depends on the presence of a basic lysine residue at position 260 (located in the juxtamembrane linker; also conserved in human). Introduction of lysine and cysteine residues at corresponding sites of STX3 (which does not have these sites) induces its palmitoylation and enhances spontaneous vesicle fusion (Vardar et al., 2022). Thus, palmitoylation can increase the fusogenic capacity of SNAREs.

Acetylation

Acetylation was first described as a histone PTM (Allfrey et al., 1964) and is probably best understood in context of genome biology. Acetylation is achieved in the cell through the donation of the acetyl group from acetyl-CoA to the α-amino group of amino acids or the ε-amino group of lysines by acetyltransferases (Narita et al., 2019). Furthermore, proteins can also undergo acetylation of free cysteines (termed S-acetylation) by members of the zDHHC acyltransferase family (Chamberlain and Shipston, 2015). Whilst acetylation is best understood in the context of histone modification, acetylation can also regulate SNARE function.

Indeed, in mammalian cells, STX17 is constitutively acetylated, and its deacetylation is required for STX17 to induce autophagy (Shen et al., 2021) (Fig. 3A). Specifically, STX17 is constitutively acetylated at lysines 219 and 223 (which are both conserved between mouse and human) by CREBBP. In starved human embryonic kidney (HEK) cells, or HEK cells treated with torin 1 (an MTORC1 inhibitor), these acetylations are removed by histone deacetylase 2 (HDAC2) (Fig. 3D). Intriguingly, HDAC2 is thought to typically localise to the nucleus (although HDAC2 has previously been observed in the cytosol; Hou et al., 2014; Milazzo et al., 2020; Xu et al., 2019). Therefore, it is unclear in this context if STX17 has to be shuttled into the nucleus (or at least to nuclear pores) in order to be deacetylated, or if HDAC2 can shuttle out of the nucleus to facilitate deacetylation. By generating acetyl-mimetic mutants of STX17 (replacing lysines 219 and 223 with glutamines), the authors demonstrate that deacetylation is not required for the recruitment of STX17 to autophagosomes but is needed for STX17-mediated autophagosome maturation (Fig. 3E). Specifically, loss of STX17 acetylation drives autophagosome–lysosome fusion through complex formation with VAMP8 and SNAP29 and interaction with the homotypic fusion and protein sorting (HOPS) complex (an assembly of proteins known as a tethering factor that enables long-range interactions between distinct membranous compartments; Fig. 3E) (Shen et al., 2021). The role of this constitutive acetylation outside of the context of autophagy is unclear; however, as in the context of autophagy, this constitutive acetylation might give the cell the ability to dynamically regulate STX17 function during biosynthetic trafficking (for example, from the ER to the Golgi).

In summary, whilst SNARE palmitoylation and acetylation are not as well studied as phosphorylation and ubiquitylation, it is clear that these modifications alter the fusogenic capacity of SNAREs and are capable of rerouting membrane traffic. Because they involve the addition of hydrophobic moieties, palmitoylation and acetylation are suited to promoting interactions between SNAREs and hydrophobic pockets of interaction partners. Thus, palmitoylation and acetylation confer SNAREs with biophysical properties that cannot be conferred by phosphorylation, ubiquitylation or SUMOylation. It will be interesting to see how the precise structures of palmitoyl and acetyl groups are utilised by the cell to promote specific SNARE–binding partner interactions.

O-GlcNAcylation

O-GlcNAcylation is a modification formed between serines and/or threonines with an N-acetylglucosamine group, an amide derivative of glucose (Yang and Qian, 2017; Zeidan and Hart, 2010). The addition and removal of O-GlcNAc groups is mediated by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively (to date only one of each enzyme has been identified). However, unlike other sugar-based modifications, O-GlcNAcylation cannot be further modified and is rapidly turned over. Thus, as is the case for phosphorylation, O-GlcNAcylation serves as a rapid on–off switch (Zeidan and Hart, 2010), but unlike phosphorylation, O-GlcNAc groups are uncharged. Thus, O-GlcNAcylation occupies a parallel niche to phosphorylation in that it rapidly alters protein function (including of SNAREs), but it does so without affecting protein charge (unlike phosphorylation).

In the context of SNARE biology, O-GlcNAcylation is best studied for SNAP29 and has been initially described as having a role in the induction of autophagy (Guo et al., 2014). O-GlcNAcylation has long been known to inhibit autophagy. Thus, to identify key proteins that are regulated by O-GlcNAcylation, the authors performed a genetic screen in *Caenorhabditis elegans* lacking OGT-1, which have an increased number of autophagosomes, and showed that the SNAREs SYX-17 (an orthologue of mammalian STX17), SNAP-29 and VAMP-8 are all required for the observed increase in autophagy. SNAP-29 was identified as the target, with O-GlcNAcylation occurring on serine 2, serine 61, threonine 130

and serine 153. Furthermore, these O-GlcNAcylations were found to block the ability of SNAP-29 to form a complex with SYX-17 and VAMP-8, and they are lost as a result of starvation (a natural autophagy inducer). These results are fascinating, as they imply that SNAP-29 normally functions whilst O-GlcNAcylated and that the loss of these O-GlcNAcylations might ensure the induction of autophagy in response to starvation (Guo et al., 2014). Although this is yet to be confirmed, the extended relevance of this work is beginning to emerge. Specifically, follow-up work has indicated that increased levels of O-GlcNAcylation (including O-GlcNAcylation of SNAP29) in heart tissue simultaneously block autophagy and exacerbate myocardial injury in a rat disease model (Huang et al., 2018). Furthermore, there is strong evidence that the toxic effects of arsenic are (in part) mediated by its effect on enhancing SNAP29 O-GlcNAcylation to block autophagy (Dodson et al., 2018).

In summary, O-GlcNAcylation is a key regulator of SNAP29 function and is likely to regulate other SNAREs. Furthermore, it is intriguing to consider the fact that only a single OGT and OGA have been identified, which suggests a more limited scope for the regulation that this modification can provide. Presumably, there are other as yet undiscovered OGTs and OGAs and/or more than one way to regulate OGT and OGA (for example, through kinases or ubiquitin ligases).

Conclusions and future directions

SNAREs are extremely well characterised in terms of their ability to confer identity to membranes and drive fusogenic events. However, it is clear that SNAREs can be modified through PTMs to modulate trafficking events, highlighting the incredible plasticity of membrane trafficking circuits. As proteomic technology continues to improve, more SNARE PTMs will likely be identified. Indeed, numerous SNARE PTMs have already been identified in high-throughput studies, many of which have not been studied further (see Table S1). For instance, the phosphorylation of STX4 at tyrosine 115 has been identified in over 200 high-throughput datasets, but its functional role is still unclear.

A number of technological challenges need to be addressed in order to comprehensively understand these PTMs. Most crucial among these is the difficulty in artificially controlling PTMs at a precise spatiotemporal level. Numerous studies have utilised mutant forms of SNAREs that cannot be posttranslationally modified. However, although these mutants may block or mimic certain SNARE PTMs, they may also interfere with other aspects of SNARE behaviour; this could alter their subcellular localisation, for instance. To that end, the use of optogenetics, for example, with caged kinase inhibitors, may prove useful in more transiently perturbing these often rapid molecular events. Indeed, in the cell migration field, light-activated proteins are already well established tools for precisely manipulating signalling events (Baarlink et al., 2017 ; Niopek et al., 2016; Wu et al., 2011). Furthermore, the precise function of specific SNARE PTMs may be difficult to ascertain, as although mutagenesis makes it possible to inhibit most PTMs, they typically do not permit biomimicry (with the exceptions of phosphorylation and acetylation). Mimicry is critical for elucidating PTMs as, arguably, blocking a molecular event through mutagenesis is easier than inducing it. Furthermore, different PTMs often target the same amino acid – for example, lysines can be ubiquitylated, SUMOylated or methylated – so mutating those residues may have a pleiotropic impact on the SNARE protein. Therefore, the use of synthetic non-natural amino acids may be required to study specific, more complex PTMs. Indeed, efforts in both mammalian and bacterial systems have already made use of synthetic amino acids to study PTMs (de la Torre and Chin, 2021), including protein sulfation (Italia et al., 2020) and acetylation (Neumann et al., 2008). Understanding of the functional effects of PTMs together with better proteomics data may also determine whether there are particular motifs that favour one type of PTM over another. For example, a more comprehensive overview of which SNAREs are modified under which conditions might reveal that certain SNARE subsets are more prone to phosphorylation, whereas others might be more susceptible to ubiquitylation.

To gain an even deeper understanding of SNARE PTMS, modelling and simulation approaches will be required. Biophysical and computational modelling has long been used to understand SNARE behaviour at a molecular level and is thus ideal for understanding SNARE PTMs (van den Bogaart et al., 2011; Risselada and Mayer, 2020). As discussed above, modifications in regulatory motifs can both promote and decrease SNARE complex formation by changing the conformation of the protein. Moreover, PTMs that are located within the core of the α-helical coiled-coil SNARE bundle can interfere with SNARE complex formation and hence membrane fusion, as has previously been demonstrated for VAMP8 (Malmersjö et al., 2016). Molecular dynamics simulations, therefore, may be useful for understanding how the location of a PTM along the SNARE affects membrane tethering and fusion. Mathematical modelling will also be important for quantitatively understanding how SNARE PTMs directly alter membrane trafficking circuits and indirectly impact a myriad of other cellular behaviours. Indeed, it is already known that altered trafficking pathways can regulate numerous cellular behaviours, including cell migration and stem cell differentiation (Derivery et al., 2015; Wilson et al., 2018). Such modelling approaches have been proven extremely successful for understanding both membrane traffic (Bezeljak et al., 2020; Vagne et al., 2020) and cellular signalling (Byrne et al., 2016; Hetmanski et al., 2016; Samaga et al., 2009), and thus may also be highly valuable for understanding SNARE PTMs.

In addition to their roles in membrane fusion, alternative, non-fusogenic functions for SNAREs have been described and will likely continue to be discovered. In the context of trafficking, this includes SNAREs that can tether membranes to each other, such as SEC22B, which is reported to tether membrane–membrane contact sites (Petkovic et al., 2014). However, the functions of SNAREs can also extend beyond a role in membrane trafficking processes; for instance, soluble STX3 can function as a transcription regulator (Giovannone et al., 2018). Therefore, it is likely that PTMs also regulate these alternative functions for SNARE proteins, or may even trigger them.

Finally, it is important to mention that there are numerous identified SNARE PTMs that are poorly understood, such as monomethylation of STX2 at lysines 55, 71 and 125 (Cao et al., 2013). As proteomics continues to improve, it is likely that many more SNARE PTMs will be found, both those that are involved in regulating membrane traffic, as well as novel ones. It will be fascinating to learn about the novel biology such modifications underpin, both at the cellular and whole-organism level, as well as their implications for health and disease.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area is supported by the European Research Council (grant 862137).

References

Allfrey, V. G., Faulkner, R. and Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis*. *Proc. Natl. Acad. Sci. USA* **51**, 786-794. https://www.pnas.org. doi:10.1073/pnas.51.5.786

Ampah, K. K., Greaves, J., Shun-Shion, A. S., Asnawi, A. W., Lidster, J. A., Chamberlain, L. H., Collins, M. O. and Peden, A. A. (2018). S-acylation regulates the trafficking and stability of the unconventional Q-SNARE STX19. *J. Cell Sci.* **131**, jcs212498. doi:10.1242/JCS.212498

Ardito, F., Giuliani, M., Perrone, D., Troiano, G. and Lo Muzio, L. (2017). The crucial role of protein phosphorylation in cell signalingand its use as targeted therapy (Review). *Int. J. Mol. Med.* **40**, 271-280. doi:10.3892/IJMM.2017.3036/HTML

Baarlink, C., Plessner, M., Sherrard, A., Morita, K., Misu, S., Virant, D., Kleinschnitz, E.-M., Harniman, R., Alibhai, D., Baumeister, S. et al. (2017). A transient pool of nuclear F-actin at mitotic exit controls chromatin organization. *Nat. Cell Biol.* **19**, 1389-1399. doi:10.1038/ncb3641

Bateman, A., Martin, M. J., Orchard, S., Magrane, M., Agivetova, R., Ahmad, S., Alpi, E., Bowler-Barnett, E. H., Britto, R., Bursteinas, B. et al. (2021). UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res.* **49**, D480-D489. doi:10.1093/NAR/GKAA1100

Bezeljak, U., Loya, H., Kaczmarek, B., Saunders, T. E. and Loose, M. (2020). Stochastic activation and bistability in a Rab GTPase regulatory network. *Proc. Natl. Acad. Sci. USA* **117**, 6504-6549. doi:10.1073/pnas.1921027117

Block, M. R., Glick, B. S., Wilcox, C. A., Wieland, F. T. and Rothman, J. E. (1988). Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. *Proc. Natl. Acad. Sci. USA* **85**, 7852. doi:10.1073/PNAS.85.21.7852

Byrne, K. M., Monsefi, N., Dawson, J. C., Degasperi, A., Bukowski-Wills, J.-C., Volinsky, N., Dobrzyński, M., Birtwistle, M. R., Tsyganov, M. A., Kiyatkin, A. et al. (2016). Bistability in the Rac1, PAK, and RhoA signaling network drives actin cytoskeleton dynamics and cell motility switches. *Cell Syst.* **2**, 38-48. doi:10.1016/J.CELS.2016.01.003

Cao, X.-J., Arnaudo, A. M. and Garcia, B. A. (2013). Large-scale global identification of protein lysine methylation in vivo. *Epigenetics* **8**, 477-485. doi:10.4161/EPI.24547

Chamberlain, L. H. and Shipston, M. J. (2015). The physiology of protein S-acylation. *Physiol. Rev.* **95**, 341-376. doi:10.1152/physrev.00032.2014

Chamberlain, S. E. L., González-González, I. M., Wilkinson, K. A., Konopacki, F. A., Kantamneni, S., Henley, J. M. and Mellor, J. R. (2012). SUMOylation and phosphorylation of GluK2 regulate kainate receptor trafficking and synaptic plasticity. *Nat. Neurosci.* **15**, 845-852. doi:10.1038/NN.3089

Chen, S. H., Shah, A. H. and Segev, N. (2011). Ypt31/32 GTPases and their F-Box effector Rcy1 regulate ubiquitination of recycling proteins. *Cellular Logist.* **1**, 21-31. doi:10.4161/CL.1.1.14695

Chou, H.-Y., Lee, Y.-T., Lin, Y. J., Wen, J.-K., Peng, W.-H., Hsieh, P.-L., Lin, S.-Y., Hung, C.-C. and Chen, G.-C. (2021). PTPN9-mediated dephosphorylation of VTI1B promotes ATG16L1 precursor fusion and autophagosome formation. *Autophagy* **17**, 2750-2765. doi:10.1080/15548627.2020.1838117

Craig, T. J., Jaafari, N., Petrovic, M. M., Rubin, P. P., Mellor, J. R. and Henley, J. M. (2012). Homeostatic synaptic scaling is regulated by protein SUMOylation. *J. Biol. Chem.* **287**, 22781-22788. doi:10.1074/JBC.M112.356337

Craig, T. J., Anderson, D., Evans, A. J., Girach, F. and Henley, J. M. (2016). SUMOylation of Syntaxin1A regulates presynaptic endocytosis. *Sci. Rep.* **5**, 17669. doi:10.1038/SREP17669

Davey, J. S., Carmichael, R. E. and Craig, T. J. (2019). Protein SUMOylation regulates insulin secretion at multiple stages. *Sci. Rep.* **9**, 2895. doi:10.1038/S41598-019-39681-6

de la Torre, D. and Chin, J. W. (2021). Reprogramming the genetic code. *Nat. Rev. Genet.* **22**, 169-184. doi:10.1038/S41576-020-00307-7

Derivery, E., Seum, C., Daeden, A., Loubéry, S., Holtzer, L., Jülicher, F. and Gonzalez-Gaitan, M. (2015). Polarized endosome dynamics by spindle asymmetry during asymmetric cell division. *Nature* **528**, 280-285. doi:10.1038/nature16443

Dingjan, I., Linders, P. T. A., Verboogen, D. R. J., Revelo, N. H., ter Beest, M. and van den Bogaart, G. (2018). Endosomal and phagosomal SNAREs. *Physiol. Rev.* **98**, 1465-1492. doi:10.1152/physrev.00037.2017

Dodson, M., Liu, P., Jiang, T., Ambrose, A. J., Luo, G., de la Vega, R., Cholanians, M., Wong, A. B., Chapman, P. K., & Zhang, E. et al. (2018). Increased O-GlcNAcylation of SNAP29 drives arsenic-induced autophagic dysfunction. *Mol. Cell. Biol.* **38**, e00595-17. doi:10.1128/MCB.00595-17

Fílla, J. and Honys, D. (2012). Enrichment techniques employed in phosphoproteomics. *Amino Acids* **43**, 1025. doi:10.1007/S00726-011-1111-Z

Francavilla, C., Rigbolt, K. T. G., Emdal, K. B., Carraro, G., Vernet, E., Bekker-Jensen, D. B., Streicher, W., Wikström, M., Sundström, M., Bellusci, S. et al. (2013). Article functional proteomics defines the molecular switch underlying FGF receptor trafficking and cellular outputs. *Mol. Cell* **51**, 707-722. doi:10.1016/j.molcel.2013.08.002

Francavilla, C., Papetti, M., Rigbolt, K. T. G., Pedersen, A. K., Sigurdsson, J. O., Cazzamali, G., Karemore, G., Blagoev, B. and Olsen, J. v. (2016). Multilayered proteomics reveals molecular switches dictating ligand-dependent EGFR trafficking. *Nat. Struct. Mol. Biol.* **23**, 608-618. doi:10.1038/NSMB.3218

Galli, S. J. and Tsai, M. (2012). IgE and mast cells in allergic disease. *Nat. Med.* **18**, 693-704. doi:10.1038/nm.2755

Giovannone, A. J., Reales, E., Bhattaram, P., Fraile-Ramos, A. and Weimbs, T. (2017). Monoubiquitination of syntaxin 3 leads to retrieval from the basolateral plasma membrane and facilitates cargo recruitment to exosomes. *Mol. Biol. Cell* **28**, 2843-2853. doi:10.1091/MBC.E17-07-0461

Giovannone, A. J., Winterstein, C., Bhattaram, P., Reales, E., Low, S. H., Baggs, J. E., Xu, M., Lalli, M. A., Hogenesch, J. B. and Weimbs, T. (2018). Soluble syntaxin 3 functions as a transcriptional regulator. *J. Biol. Chem.* **293**, 5478-5491. doi:10.1074/JBC.RA117.000874

Guo, B., Liang, Q., Li, L., Hu, Z., Wu, F., Zhang, P., Ma, Y., Zhao, B., Kovács, A. L., Zhang, Z. et al. (2014). O-GlcNAc-modification of SNAP-29 regulates autophagosome maturation. *Nat. Cell Biol.* **16**, 1215-1226. doi:10.1038/ncb3066

Hamasaki, M., Furuta, N., Matsuda, A., Nezu, A., Yamamoto, A., Fujita, N., Oomori, H., Noda, T., Haraguchi, T., Hiraoka, Y. et al. (2013). Autophagosomes form at ER–mitochondria contact sites. *Nature* **495**, 389-393. doi:10.1038/nature11910

He, Y. and Linder, M. E. (2009). Differential palmitoylation of the endosomal SNAREs syntaxin 7 and syntaxin 8. *J. Lipid Res.* **50**, 398-404. doi:10.1194/JLR.M800360-JLR200

Henley, J. M., Craig, T. J. and Wilkinson, K. A. (2014). Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction. *Physiol. Rev.* **94**, 1249-1285. doi:10.1152/physrev.00008.2014

Hernandez, J. M., Stein, A., Behrmann, E., Riedel, D., Cypionka, A., Farsi, Z., Walla, P. J., Raunser, S. and Jahn, R. (2012). Membrane fusion intermediates via directional and full assembly of the SNARE complex. *Science* **336**, 1581-1584. doi:10.1126/SCIENCE.1221976

Hetmanski, J. H. R., Zindy, E., Schwartz, J.-M. and Caswell, P. T. (2016). A MAPK-driven feedback loop suppresses Rac activity to promote RhoA-driven cancer cell invasion. *PLoS Comput. Biol.* **12**, e1004909. doi:10.1371/JOURNAL.PCBI.1004909

Hornbeck, P. V., Zhang, B., Murray, B., Kornhauser, J. M., Latham, V. and Skrzypek, E. (2015). PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res.* **43**, D512-D520. doi:10.1093/NAR/GKU1267

Hou, N., Gong, M., Bi, Y., Zhang, Y., Tan, B., Liu, Y., Wei, X., Chen, J. and Li, T. (2014). Spatiotemporal expression of HDAC2 during the postnatal development of the rat hippocampus. *Int. J. Med. Sci.* **11**, 788-795. doi:10.7150/IJMS.8417

Huang, S., Tang, D. and Wang, Y. (2016). Monoubiquitination of Syntaxin 5 regulates Golgi membrane dynamics during the cell cycle. *Dev. Cell* **38**, 73-85. doi:10.1016/J.DEVCEL.2016.06.001

Huang, L., Yuan, P., Yu, P., Kong, Q., Xu, Z., Yan, X., Shen, Y., Yang, J., Wan, R., Hong, K. et al. (2018). O-GlcNAc-modified SNAP29 inhibits autophagy-mediated degradation via the disturbed SNAP29-STX17-VAMP8 complex and exacerbates myocardial injury in type I diabetic rats. *Int. J. Mol. Med.* **42**, 3278-3290. doi:10.3892/IJMM.2018.3866

Italia, J. S., Peeler, J. C., Hillenbrand, C. M., Latour, C., Weerapana, E. and Chatterjee, A. (2020). Genetically encoded protein sulfation in mammalian cells. *Nat. Chem. Biol.* **16**, 379-382. doi:10.1038/S41589-020-0493-1

Jahn, R. and Scheller, R. H. (2006). SNAREs — engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* **7**, 631-643. doi:10.1038/nrm2002

Kang, R., Wan, J., Arstikaitis, P., Takahashi, H., Huang, K., Bailey, A. O., Thompson, J. X., Roth, A. F., Drisdel, R. C., Mastro, R. et al. (2008). Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature* **456**, 904-909. doi:10.1038/NATURE07605

Karim, Z. A., Zhang, J., Banerjee, M., Chicka, M. C., al Hawas, R., Hamilton, T. R., Roche, P. A. and Whiteheart, S. W. (2013). IκB kinase phosphorylation of SNAP-23 controls platelet secretion. *Blood* **121**, 4567-4574. doi:10.1182/BLOOD-2012-11-470468

Kliza, K. and Husnjak, K. (2020). Resolving the complexity of ubiquitin networks. *Front. Mol. Biosci.* **7**, 21. doi:10.3389/fmolb.2020.00021

Kumar, S., Gu, Y., Abudu, Y. P., Bruun, J. A., Jain, A., Farzam, F., Mudd, M., Anonsen, J. H., Rusten, T. E., Kasof, G. et al. (2019). Phosphorylation of syntaxin 17 by TBK1 controls autophagy initiation. *Dev. Cell* **49**, 130-144.e6. doi:10.1016/J.DEVCEL.2019.01.027

Linder, M. E. and Deschenes, R. J. (2007). Palmitoylation: policing protein stability and traffic. *Nat. Rev. Mol. Cell Biol.* **8**, 74-84. doi:10.1038/nrm2084

Malhotra, V., Orci, L., Glick, B. S., Block, M. and Rothman, J. E. (1988). Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the golgi stack. *Cell* **54**, 221-227. doi:10.1016/0092-8674(88)90554-5

Malmersjö, S., Palma, S. d., Diao, J., Lai, Y., Pfuetzner, R. A., Wang, A. L., McMahon, M. A., Hayer, A., Porteus, M., Bodenmiller, B. et al. (2016). Phosphorylation of residues inside the SNARE complex suppresses secretory vesicle fusion. *EMBO J.* **35**, 1810-1821. doi:10.15252/EMBJ.201694071

Matsuoka, K., Morimitsu, Y., Uchida, K. and Schekman, R. (1998). Coat assembly directs v-SNARE concentration into synthetic COPII vesicles. *Mol. Cell* **2**, 703-708. doi:10.1016/S1097-2765(00)80168-9

Matsuoka, S., Ballif, B. A., Smogorzewska, A., McDonald, E. R., Hurov, K. E., Luo, J., Bakalarski, C. E., Zhao, Z., Solimini, N., Lerenthal, Y. et al. (2007).

ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**, 1160-1166. doi:10.1126/SCIENCE.1140321

McGrath, K., Agarwal, S., Tonelli, M., Dergai, M., Gaeta, A. L., Shum, A. K., Lacoste, J., Zhang, Y., Wen, W., Chung, D. et al. (2021). A conformational switch driven by phosphorylation regulates the activity of the evolutionarily conserved SNARE Ykt6. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2016730118. doi:10.1073/pnas.2016730118

Milazzo, G., Mercatelli, D., di Muzio, G., Triboli, L., de Rosa, P., Perini, G. and Giorgi, F. M. (2020). Histone deacetylases (HDACs): evolution, specificity, role in transcriptional complexes, and pharmacological actionability. *Genes* **11**, 556. doi:10.3390/GENES11050556

Muppirala, M., Gupta, V. and Swarup, G. (2011). Syntaxin 17 cycles between the ER and ERGIC and is required to maintain the architecture of ERGIC and Golgi. *Biol. Cell* **103**, 333-350. doi:10.1042/BC20110006

Nair-Gupta, P., Baccarini, A., Tung, N., Seyffer, F., Florey, O., Huang, Y., Banerjee, M., Overholtzer, M., Roche, P. A., Tampé, R. et al. (2014). TLR signals induce phagosomal MHC-I delivery from the endosomal recycling compartment to allow cross-presentation. *Cell* **158**, 506-521. doi:10.1016/J.CELL.2014.04.054

Narita, T., Weinert, B. T. and Choudhary, C. (2019). Functions and mechanisms of non-histone protein acetylation. *Nat. Rev. Mol. Cell Biol.* **20**, 156-174. doi:10.1038/S41580-018-0081-3

Neumann, H., Peak-Chew, S. Y. and Chin, J. W. (2008). Genetically encoding Nε-acetyllysine in recombinant proteins. *Nat. Chem. Biol.* **4**, 232-234. doi:10.1038/NCHEMBIO.73

Niopek, D., Wehler, P., Roensch, J., Eils, R. and di Ventura, B. (2016). Optogenetic control of nuclear protein export. *Nat. Commun.* **7**, 10624. doi:10.1038/ncomms10624

Olsen, J. v., Vermeulen, M., Santamaria, A., Kumar, C., Miller, M. L., Jensen, L. J., Gnad, F., Cox, J., Jensen, T. S., Nigg, E. A. et al. (2010). Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci. Signal.* **3**, 104. doi:10.1126/SCISIGNAL.2000475

Patente, T. A., Pinho, M. P., Oliveira, A. A., Evangelista, G. C. M., Bergami-Santos, P. C. and Barbuto, J. A. M. (2019). Human dendritic cells: their heterogeneity and clinical application potential in cancer immunotherapy. *Front. Immunol.* **9**, 3176. doi:10.3389/FIMMU.2018.03176

Petkovic, M., Jemaiel, A., Daste, F., Specht, C. G., Izeddin, I., Vorkel, D., Verbavatz, J.-M., Darzacq, X., Triller, A., Pfenninger, K. H. et al. (2014). The SNARE Sec22b has a non-fusogenic function in plasma membrane expansion. *Nat. Cell Biol.* **16**, 434-444. doi:10.1038/ncb2937

Pradhipa Karuna, M., Witte, L., Linnemannstoens, K., Choezom, D., Danieli-Mackay, A., Honemann-Capito, M. and Gross, J. C. (2020). Phosphorylation of Ykt6 SNARE domain regulates its membrane recruitment and activity. *Biomolecules* **10**, 1560. doi:10.3390/BIOM10111560

Prakash, K. and Fournier, D. (2017). Histone code and higher-order chromatin folding: a hypothesis. *Genomics Comput. Biol.* **3**, 41. doi:10.18547/GCB.2017.VOL3.ISS2.E41

Prekeris, R., Yang, B., Oorschot, V., Klumperman, J. and Scheller, R. H. (1999). Differential roles of syntaxin 7 and syntaxin 8 in endosomal trafficking. *Mol. Biol. Cell* **10**, 3891-3908. doi:10.1091/MBC.10.11.3891

Puri, N. and Roche, P. A. (2006). Ternary SNARE complexes are enriched in lipid rafts during mast cell exocytosis. *Traffic* **7**, 1482-1494. doi:10.1111/J.1600-0854.2006.00490.X

Risselada, H. J. and Mayer, A. (2020). SNAREs, tethers and SM proteins: how to overcome the final barriers to membrane fusion? *Biochem. J.* **477**, 243-258. doi:10.1042/BCJ20190050

Samaga, R., Saez-Rodriguez, J., Alexopoulos, L. G., Sorger, P. K. and Klamt, S. (2009). The logic of EGFR/ErbB signaling: theoretical properties and analysis of high-throughput data. *PLoS Comput. Biol.* **5**, e1000438. doi:10.1371/JOURNAL.PCBI.1000438

Shalizi, A., Gaudillière, B., Yuan, Z., Stegmüller, J., Shirogane, T., Ge, Q., Tan, Y., Schulman, B., Harper, J. W. and Bonni, A. (2006). A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. *Science* **311**, 1012-1017. doi:10.1126/SCIENCE.1122513

Sharma, K., D'Souza, R. C. J., Tyanova, S., Schaab, C., Wiśniewski, J. R., Cox, J. and Mann, M. (2014). Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. *Cell Rep.* **8**, 1583-1594. doi:10.1016/J.CELREP.2014.07.036

Shen, Q., Shi, Y., Liu, J., Su, H., Huang, J., Zhang, Y., Peng, C., Zhou, T., Sun, Q., Wan, W. et al. (2021). Acetylation of STX17 (syntaxin 17) controls autophagosome maturation. *Autophagy* **17**, 1157-1169. doi:10.1080/15548627.2020.1752471

Sneeggen, M., Pedersen, N. M., Campsteijn, C., Haugsten, E. M., Stenmark, H. and Schink, K. O. (2019). WDFY2 restrains matrix metalloproteinase secretion and cell invasion by controlling VAMP3-dependent recycling. *Nat. Commun.* **10**, 2850. doi:10.1038/S41467-019-10794-W

Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J. E. (1993). SNAP receptors implicated in vesicle targeting and fusion. *Nature* **362**, 318-324. doi:10.1038/362318a0

Suzuki, K. and Verma, I. M. (2008). Phosphorylation of SNAP-23 by IκB kinase 2 regulates mast cell degranulation. *Cell* **134**, 485-495. doi:10.1016/J.CELL.2008.05.050

Vagne, Q., Vrel, J.-P. and Sens, P. (2020). A minimal self-organisation model of the golgi apparatus. *ELife* **9**, e47318. doi:10.7554/ELIFE.47318

Valdez-Taubas, J. and Pelham, H. (2005). Swf1-dependent palmitoylation of the SNARE Tlg1 prevents its ubiquitination and degradation. *EMBO J.* **24**, 2524-2532. doi:10.1038/SJ.EMBOJ.7600724

van den Bogaart, G., Meyenberg, K., Risselada, H. J., Amin, H., Willig, K. I., Hubrich, B. E., Dier, M., Hell, S. W., Grubmüller, H., Diederichsen, U. et al. (2011). Membrane protein sequestering by ionic protein-lipid interactions. *Nature* **479**, 552-555. doi:10.1038/NATURE10545

Vardar, G., Salazar-Lázaro, A., Zobel, S., Trimbuch, T. and Rosenmund, C. (2022). Syntaxin-1A modulates vesicle fusion in mammalian neurons via juxtamembrane domain dependent palmitoylation of its transmembrane domain. *ELife* **11**, e78182. doi:10.7554/ELIFE.78182

Verboogen, D. R. J., Revelo, N. H., ter Beest, M. and van den Bogaart, G. (2019). Interleukin-6 secretion is limited by self-signaling in endosomes. *J. Mol. Cell Biol.* **11**, 144-157. doi:10.1093/JMCB/MJY038

Weber, T., Zemelman, B. v., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T. H. and Rothman, J. E. (1998). SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759-772. doi:10.1016/S0092-8674(00)81404-X

Wilkinson, K. A. and Henley, J. M. (2010). Mechanisms, regulation and consequences of protein SUMOylation. *Biochem. J.* **428**, 133-145. doi:10.1042/BJ20100158

Wilson, B. J., Allen, J. L. and Caswell, P. T. (2018). Vesicle trafficking pathways that direct cell migration in 3D matrices and in vivo. *Traffic* **19**, 899. doi:10.1111/TRA.12605

Wu, Y. I., Wang, X., He, L., Montell, D. and Hahn, K. M. (2011). Spatiotemporal control of small GTPases with light using the LOV domain. *Methods Enzymol.* **497**, 393-407. doi:10.1016/B978-0-12-385075-1.00016-0

Xu, P., Hankins, H. M., MacDonald, C., Erlinger, S. J., Frazier, M. N., Diab, N. S., Piper, R. C., Jackson, L. P., MacGurn, J. A. and Graham, T. R. (2017). COPI mediates recycling of an exocytic SNARE by recognition of a ubiquitin sorting signal. *ELife* **6**, e28342. doi:10.7554/ELIFE.28342

Xu, P., Ye, S., Li, K., Huang, M., Wang, Q., Zeng, S., Chen, X., Gao, W., Chen, J., Zhang, Q. et al. (2019). NOS1 inhibits the interferon response of cancer cells by S-nitrosylation of HDAC2. *J. Exp. Clin. Cancer Res.* **38**, 483. doi:10.1186/S13046-019-1448-9

Yang, X. and Qian, K. (2017). Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* **18**, 452-465. doi:10.1038/NRM.2017.22

Zeidan, Q. and Hart, G. W. (2010). The intersections between O-GlcNAcylation and phosphorylation: implications for multiple signaling pathways. *J. Cell Sci.* **123**, 13-22. doi:10.1242/JCS.053678